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PARADAXIN'S ACTION IN SHARK(U) NEW YORK AQUARIUM  
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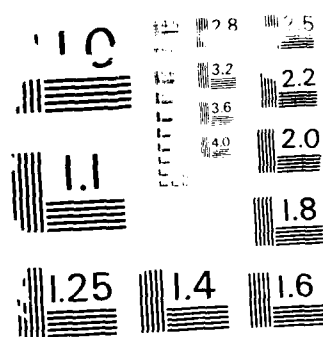
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NEW YORK AQUARIUM and

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February 26, 1988

Dr. Michael T. Marron  
Scientific Officer, Biotechnology  
Department of the Navy  
Office of Naval Research  
Code 1141 MB  
800 North Quincy Street  
Arlington, Virginia 22217-5000

Dear Dr. Marron:

A Progress Report for the contract N00014-82-C-0435  
is submitted.

*JAN. - Dec 1987*

Sincerely,

Naftali Primor  
Principal Investigator

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# PARDAXIN'S ACTION IN SHARK

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Osborn Laboratories of Marine Sciences

ASSISTED BY Dr. Ychiel, Dr. Jay Fox, Dr. Charles Edwards

WORK UNIT NO. NR

CONTRACT N00014-82-C-0435

*The objectives of this research were:*  
OBJECTIVES

1. In Vivo binding study of pardaxin (PX) to gill membranes.
2. Testing for activities of a synthetic pardaxin and N-terminal decapeptide and 26 amino acid peptide. and
3. Permeabilities for monovalent and divalent cations in pardaxin channels in lipid bilayer membranes.

## ABSTRACT

1. In experiments in which we tested the effect of proteinase K (PK) and pronase on PX binding and toxicity, the enzyme was first added to the medium for 20 min. Then the fish were washed and <sup>3</sup>H-PX with and without unlabelled PX was added to the medium for 30 min. The effect of unlabelled PX on the binding of <sup>3</sup>H-PX and toxicity is given in the table below.

CPM/FISH  
(mean)

TOXICITY  
(%)

<sup>3</sup> H-PX	230	0
<sup>3</sup> H-PX + PX (25 ug/mL)	212	0
<sup>3</sup> H-PX + PX (50 ug/mL)	490	30
Treated with PK		
<sup>3</sup> H-PX	260	0
<sup>3</sup> H-PX + PX (25 ug/mL)	512	40
Treated with pronase		
<sup>3</sup> H-PX	230	0
<sup>3</sup> H-PX + PX (25 ug/mL)	222	0

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These results indicate that:

The exposure of fish to PK, but not to pronase, enhanced PX toxicity and binding. Fish treated with PK responded to 25 ug/mL of PX as to 50 ug/ml with no previous exposure to PK.

Upon the addition of unlabelled PX (50 ug/mL) no displacement was observed, indicating that no competitive binding occurred.

2. PX N-terminal decapeptide and the 26 amino acids peptide were synthesized by the solid phase method and were tested for the following activities:

- a. Effect on fish at a concentration of 25 ug/mL PX-33 - causes jumps in a swimming fish. The PX-10 and PX-26 failed to produce such an effect at 400 ug/mL.
- b. Effect on release of neurotransmitter - In frog neuromuscular preparation, at 5-10 ug/mL PX-33 produced an increase in ACH release. The PX-10 and PX-26 were inactive at 200 ug/mL.
- c. Channel formation in liposomes - This effect is produced at nanogram concentrations of PX-33. The PX-10 and PX-26 could not produce channels at 100 times higher concentration.
- d. Aggregation of small liposomes - This is the only effect that the PX-10 and PX-26 peptides elicited, but only at concentrations 10 times greater than that of PX-33.

We concluded that the biological activities of PX-33 are not caused by high hydrophobicity (detergent effect) alone, and that a larger portion than 26 amino acids is needed to retain its activity.

3. We determined the primary sequence of pardaxin:

10

NH<sub>2</sub> - Gly-Phe-Phe-Ala-Leu-Ile-Pro-Gly-Lys-Ile-Ser-Ser-Pro-Ile-Phe-Lys-Thr-Leu-Leu-Ser-Ala-Val-Gly-Ser-Ala-Leu-Ser-Ser-Ser-GL̄y-Gly-Gln-Glu - COOH      20                          30

Its composed of two distinct regions: a very hydrophobic region of 26 amino acids and 7 N-terminal hydrophilic acids.

#### 4. PERMEABILITY RATIOS FOR MONOVALENT AND DIVALENT CATIONS IN PARDAXIN CHANNELS IN LIPID BILAYER MEMBRANES

ION	$P_I/P_K$	RELATIVE HYDRATED SIZE
Rb <sup>+</sup>	1.19	0.95
Cs <sup>+</sup>	1.15	0.94
NH <sub>4</sub> <sup>+</sup>	1.03	1.0
Guanidine	1.01	
K <sup>+</sup>		1.0
Li <sup>+</sup>	0.75	1.90
Methylamine	0.69	
Na <sup>+</sup>	0.63	1.46
Dimethylamine	0.45	

PI/Mg

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Ba++	1.29	0.83
Sr++	1.26	0.88
Mn++	1.18	1.02
Mg++	1.0	1.00
Ethylenediamine	0.99	

LOWEST CONCENTRATION REQUIRED TO  
INDUCE SINGLE CHANNEL ACTIVITY

PX-33      0.25 ug/ml  
PX-26      100 ug/ml  
PX-10      no effect at  
            1750 ug/ml

Published work on the subject:

Renner, P., C. G. Caratsch, P. G. Waser, P. Lazarovici and N. Primor.  
Presynaptic effects of the pardaxins, polypeptides isolated from  
the gland secretion of the flatfish Pardachiras marmoratus.  
Neuroscience 23, 319-325, 1987.

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